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PHYLOGENETIC RELATIONSHIPS OF ENTOMOPATHOGENIC NEMATODES AND THEIR BACTERIAL SYMBIONTS FROM COASTAL AREAS IN LEBANON

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Noujeim E., Sakr J., Fanelli E., Troccoli A., Pages S., Tarasco E., De Luca F. – Phylogenetic relationships of entomopathogenic nematodes and their bacterial symbionts from coastal areas in Lebanon.

Entomopathogenic nematodes (EPNs) are parasites of soil-dwelling insects that occur in natural and agricultural soils around the world. The current study focuses on the unexplored coastal zone of Lebanon where soil samples were taken in different sites chosen randomly along the coast like beaches, agricultural and herbaceous fields. In total, 350 soil samples were collected, mainly from the southern part of the country. An integrated approach, combining both traditional (morphological) and molecular methods, was used to characterize entomopathogenic nematode species encountered. Two named-species are added to the EPNs catalog in Lebanon from 4 samples out of the total 350 samples isolated: *Heterorhabditis indica*, reported for the first time in the country (samples AYAB6 and BRA20) and *Steinernema feltiae* (samples ANFA5 and EDA1). Furthermore, one undescribed potential entomopathogenic nematode belonging to *Oscheius* genus was recovered. The symbiotic bacteria from *S. feltiae* and *H. indica* were also molecularly identified through the use of five gene fragments *recA*, *gyrB*, *dnaN*, *gltX* and *infB*. Phylogenetic relationships of entomopathogenic nematodes and their symbiotic bacteria were inferred by using maximum-likelihood analysis. Soil studies were subsequently carried out in order to assess a possible relationship between soil parameters and their effects on EPNs. Results indicate that sandy texture and moisture are key factors for the presence and survival of EPNs in the soil in Lebanon.

KEY WORDS**:** survey, entomopathogens, nematode, Lebanon, symbiotic bacteria, phylogeny.

INTRODUCTION

EPNs from Steinernematidae and Heterorhabditidae families are a widespread component of most terrestrial ecosystems and recorded from all continents with the exception of Antarctica (HOMINICK, 2002). They are obligate parasites of insects harbouring mutualistic bacterial symbionts (*Xenorhabdus* spp. for Steinernematidae and *Photorhabdus* spp. for Heterorhabditidae) that kill the host and digest its tissues, providing food necessary for completing nematode growth and development inside the insect host (NADLER *et al*., 2006). The infective juvenile stages (IJs) penetrates the hemocoel of the insect and release their symbiotic bacteria into the hemolymph which contribute to killing by septicemia of the insect host in 24 to 72 hours (FORST and CLARKE, 2002). The IJs of entomopathogenic nematodes (EPN) are of great interest because of their potential in regulating insect populations and, therefore, as biological control agents of many insect pests. They are found in a variety of soil habitats and their mobility and persistence in the soil may be influenced by numerous factors, such as soil moisture, soil texture, vegetation cover and insect hosts (MOLYNEUX and BEDDING, 1984; KUNG *et al*., 1991; KOPPENHOFER *et al*., 1995; GRANT and VILLANI, 2003a; 2003b; TARASCO *et al*., 2015). During the first prospection of entomopathogenic nematodes conducted in the different vegetation levels in Lebanon (NOUJEIM *et al*., 2011), two EPN species were recovered: *Steinernema feltiae* (Filipjev, 1934) (WOUTS *et al*., 1982) and *Heterorhabditis bacteriophora* (POINAR, 1976) in mountainous sites characterized by sandy soils, in the vicinity of water sources. In light of these results, the current study has focused on the survey of the coastal area of Lebanon, particularly the southern side of the country. In the present study, *Steinernema feltiae* and *Heterorhabditis indica* (POINAR, KARUNAKAR and DAVID 1992) were recovered and characterized at morphological and molecular level. A potential entomopathogenic nematode species belonging to genus *Oscheius* was also found and characterized at molecular level. Nematodes belonging to *Oscheius* genus are free-living nematodes living in the soil showing various associations with invertebrate hosts, ranging from necromenic to facultative and occasionally obligate parasitism. The corresponding bacterial symbionts of *S. feltiae* and *H. indica* were also identified and a reliable phylogenetic tree was obtained by the multigene approach from a set of concatenated partial sequences from five gene fragments *recA*, *gyrB*, *dnaN*, *gltX* and *infB*.

MATERIAL AND METHODS

STUDY AREA

Situated on the eastern side of the Mediterranean Sea, Lebanon extends over 220 Kilometers from the North till the South and over 48 Kilometers from the West to the East. The coastal zone, comprised between the Mount Lebanon

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chain and the Mediterranean Sea, is characterized by being very narrow, except in the north and the south of the country. In a 500 m-wide corridor along the coastline, coastal zones represent eight percent of the total Lebanese surface area, which is approximately 840 km^2 of the Lebanese territories. The mean annual temperature on the coastal zone varies between 13.5 and 27°C with an average annual rainfall of 600 mm. The coastline is strongly heavily cut and is marked with a series of promontories, cliffs and bays. The coastline is well-known for housing natural habitats for endangered fauna and flora (CDR, 2005; MoE, 2005). Rocky, sandy, silty, coastal, neritic and oceanic habitats are present along this line with a dominance of sandy habitats along the South side of the country and rocky habitats along the North side. The presence of big, highly urbanized cities further reduces the presence of natural habitats by replacing them with polluted ecosystems**.**

Collection and characterization of soil samples

Along the coastal area of Lebanon, with a particular focus on the southern side of the country, different habitats were selected, covering natural and cultivated ecosystems ranging from 0 to 200 m above sea level, from three main habitats (which are the most commonly encountered habitat): orchard, seacoast and grassland. Within each site, 10 soil samples were collected in an area of 50-100 m^2; each soil sample (approximately 1kg) consisted of 3-5 sub-samples mixed in one sample, taken randomly, at a depth of 0–20 cm. Samples were taken with a hand shovel, placed in polyethylene bags to prevent water loss, kept in coolers during transit to the laboratory. For each positive site, random soil sub-samples were pooled and the composite soil sample was sieved (2-mm) and air-dried. Soil pH was measured in a water suspension (soil: water ratio of 1:2.5, w/v) (Thomas, 1996). Besides organic matter, soil texture and relative humidity were measured for soil samples resulting positive or negative to the presence of EPNs.

Isolation of EPNs from soil

EPNs were isolated by *Galleria mellonella* L. bait method (Bedding and Akhurst, 1975). Soil samples were placed in plastic containers and one steel mesh pocket containing three last-instar *G. mellonella* (Lepidoptera: Pyralidae) larvae was placed in each container and kept at room temperature in the Nematology laboratory of CNRS-Lebanon. Juveniles emerging from *Galleria* cadavers were collected and kept in a laboratory culture in 50 ml Ringer solution. Part of them was sent to the Institute of Sustainable Plant Protection (IPSP)-CNR in Bari for morphological and molecular characterization.

Morphological characterization

For morphological studies, nematodes were examined live or heat-killed in 60°C Ringer solution. The heat-killed nematodes were placed in triethanolamine–formalin (TAF) fixative (Kaya and Stock, 1997) and processed to anhydrous glycerine for mounting (Hooper, 1970). Measurements of infective juveniles were taken on freshly mounted specimens, while male measurements were taken on permanently mounted, glycerin infiltrated specimens. Observations were made using a Leitz Diaplan optical microscope equipped with differential interference contrast optics and Leica® DFC425 camera. Measurements of specimens were made using LAS (Leica Application Suite) Version 3.6.0 software. For morphological characterization of the isolates, morpho-diagnostic characters of 10 males and 10 IJs, for each strain, were studied.

DNA extraction, PCR amplification and sequencing

Specimens of EPNs for molecular analysis were kept in Ringer solution. Genomic DNA was extracted from fifteen individual nematodes as described by De Luca *et al*. (2004). The crude DNA isolated from each individual nematode was directly amplified. The ITS1-5.8S-ITS2 regions were amplified using the forward primer TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and the reverse primer AB28 (5'-ATATGCTTAAGTTCAGCGGGT -3') (Subbotin *et al.*, 2001); the 18S rDNA was amplified using the 18SnF (5'-TGGATAACTGTGGTAATTCTAGAGC-3') and 18SnR (5'-TTACGACTTTTGCCCGGTTC-3') the portion of the mitochondrial cytochrome oxidase c subunit 1 (*mt*COI) gene was amplified with this primer set: COI-F1 (5'-CCTACTATGATTGGTGGTTTTGGTAATTG-3') and COI-R2 (5'-GTAGCAGCAGTAAAATAAGCACG-3') (Kanzaki and Futai, 2002).

Amplification conditions were an initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C/50s, 55°C/50s, 72°C/1 min, with a final extension time of 7 min at 72°C. All products were examined by standard electrophoresis on a 1% agarose gel. PCR products from two individual nematodes for each population were purified for sequencing using the protocol listed by manufacturer (High Pure PCR elution kit, Roche, Germany). Purified DNA fragments were cloned and sequenced in both directions. A BLAST (Basic Local Alignment Search Tool) search at NCBI (National Center for Biotechnology Information) was performed to confirm their nematode origins and species (Altschul *et al*., 1997). The newly obtained sequences for ITS, 18SrRNA gene and COI were aligned using ClustalW (Larkin *et al*., 2007) with default parameters using corresponding published gene sequences of *Heterorhabditis*, *Steinernema* and *Oscheius*. Sequence alignments were manually edited using BioEdit in order to improve the default parameters of the multialignment. Outgroup taxa for each dataset were chosen according to the results of previously published data. Phylogenetic trees, obtained for ITS, 18SrRNA gene and mitochondrial COI, were performed with Neighbour-Joining (NJ), Minimum Evolution (ME), Maximum Parsimony (MP) and Maximum Likelihood (ML) methods using MEGA version 6 software (Tamura *et al*., 2007). No significant conflict in branching order and support level among methods is observed and, therefore, only ML tree is shown for each marker.

The phylograms were bootstrapped 1,000 times to assess the degree of support for the phylogenetic branching indicated by the optimal tree for each method. All sequences determined in this study have been deposited at GenBank. The accession numbers are listed in Table 1.

Bacterial symbiont isolation and identification

The symbiotic bacteria of the entomopathogenic nematodes were isolated from hemolymph of an infected *G. mellonella* cadaver by plating on nutrient agar supplemented with 0.004% (w/v) triphenyltetrazolium chloride and 0.0025% (w/v) bromothymol blue (NBTA medium) at 28°C for 48 h (Boemare *et al*., 1997).

The phylogenetic classification of the new bacteria was based on a multigene approach including five universally conserved protein-coding sequences (*recA, gyrB, dnaN, gltX* and *infB*) obtained as previously described (Tailliez *et al*., 2010; 2012). For each bacterial isolates, individual gene fragments (*recA*, 646 nucleotides; *gyrB*, 864 nucleotides; *dnaN*, 828 nucleotides; *gltX*, 1057 nucleotides and *infB*, 965

Table 1 – Isolated entomopathogenic nematodes and bacteria according to sampled region and ecosystem.

Species name	EPN sample	Gene marker	Accession number	Species name	Symbiotic bacteria sample	Country	Ecosystem
Heterorhabditis indica	AYAB6	COI	LM608086-LM608087	*Photorhabdus luminescens* subsp. *akhurstii*	LB06	Aytamoun	Agriculture (Banana)
		ITS	LN611141				
		18S	LN611143				
	BRA20	ITS	LN611140	*Photorhabdus luminescens* subsp. *akhurstii*	LB20	Burj Rahal	Agriculture (Banana)
		18S	LN611144				
Steinernema feltiae	ANFA5	COI	LM608089-LM608090	*Xenorhabdus bovienii*	LB05	Anfeh	Agriculture (Olive)
		ITS	LN611136-LN611137				
		18S	LN611146-LN611147				
	EDA1	COI	LM608088	*Xenorhabdus bovienii*	LB10	Edde	Agriculture (Potatoes)
		ITS	LN611138-LN611139				
		18S	LN611148				
Oscheius sp.	BRA6	COI	LM608091			Burj Rahal	Agriculture (Orange)
		ITS	LN611142				

nucleotides) were aligned using MUSCLE (Edgar, 2004) and then concatenated using the SeaView platform (http://pbil.univ-lyon1.fr/software/seaview.html). Ambiguously aligned blocks were removed using the Gblocks method (Castresana, 2000). The maximum-likelihood analysis (phyML 3.0) was carried out with the general time reversible model of substitution with gamma-distributed rate heterogeneity and a proportion of invariant sites determined for all five protein-coding sequences by jModelTest to best fit with the data using the AIC criterion (Posada and Crandall, 1998). MUSCLE, Gblocks and PhyML were obtained from the phylogeny.fr platform (Dereeper *et al*., 2008). The sequences of the bacterial isolates were compared with those of representative strains of 29 *Xenorhabdus* species and 21 *Photorhabdus* species/subspecies. TheGenBank/EMBL/DDBJ accession numbers for the partial 16S rRNA gene, *recA, gyrB, dnaN, gltX* and *infB* sequences of strain LB05 are LN835363, and LN835358-LN835362, respectively, those for strain LB10 are LN835364, and LN835353-LN835357, respectively, and those for strain LB06 are LN835365 and LN835348-LN835352, respectively.

RESULTS

Presence of EPNs along the coastal area

EPNs were found in three different coastal ecosystems: herbaceous, agricultural and sandy beaches (Table 1). Out of the 350 samples taken, 20 samples contained nematodes, but only four strains were identified as EPNs. Aytamoun site is a banana field with a sandy loam soil slightly alkaline (pH=8.58) where *H. indica* (strain AYAB6) was isolated. Another population of *H. indica* strain BRA20 was also isolated in the South of the country (Burj Rahal) in a banana field. Along the northern side of the country *S. feltiae* (ANFA5) was recovered from an olive orchard with a high percentage of clay 30.72% and a low percentage of organic matter in the soil (2.63%). *Steinernema feltiae* EDA1 was also recovered from a potato field in the North of the country. In addition to EPNs, we isolated a nematode belonging to the genus *Oscheius* recovered in an orange grove BRA6 (Table 3).

Morphological characterization

Measurements of main diagnostic characters of infective juveniles and of first generation (G1) males of *S. feltiae* (EDA1 and ANFA5 populations) and *H. indica* (AYAB6 and BRA20) are reported in Table 2. In general, morphometrics of Lebanese strains is in agreement with data from literature. Few exceptions concerned a wider body diameter of males of *S. feltiae* ANFA5 population (101-126 *vs* 60-90 µm of original description) and a larger spicule length/anal body width (SL/ABW) ratio in both *S. feltiae* Lebanese populations (1.4-2.0 and 1.6-2.1 in ANFA5 and EDA1 populations, respectively, *vs* 0.99-1.3 of original description) (Wouts *et al*., 1982).

Sequence analyses of the ribosomal and mitochondrial regions

The DNA sequences of ITS region of *S. feltiae* were determined for 2 specimens for each population and produced a sequence of 808 bp long (ANFA5 and EDA1). No intraspecific differences were found in the sequences of these specimens. The ITS sequences of *S. feltiae* from Lebanon showed a intra-specific variability with the

Table 2 – Morphometrics of fresh-heat killed (water-agar temporary mounts) infective juveniles (IJs) and glycerin infiltrated first generation males (G1) of EPN populations from coastal areas of Lebanon. Measurements are in µm, in the form: mean ± S.D. (range).

	EDA1 IJs	EDA1 G1 Males	ANFA5 IJs	ANFA5 G1 Males	AYAB6 IJs	AYAB6 G1 Males
L	898 ± 51.1 (813.0-985.3)	1151 ± 70.2 (1042-1315)	915 ± 33.1 (857-956)	1476 ± 76.6 (1388-1574)	632.5 ± 20.9 (601-665)	892.0 ± 52.6 (823-1016)
a	30.5 ± 1.6 (27.8-33.7)	18.8 ± 2.3 (15.2-23.4)	30.4 ± 2.5 (25.4-33.5)	12.9 ± 0.9 (15.2-23.4)	26 ± 1.5 (23.3-28.4)	18.8 ± 1.1 (16.8-20.8)
b	7.0 ± 0.9 (6.1-9.4)	8.4 ± 0.4 (7.6-9.2)	6.4 ± 0.4 (5.5-6.9)	10.5 ± 0.9 (8.9-11.5)	4.9 ± 0.1 (4.6-5.0)	8.3 ± 0.4 (7.9-9.2)
c	10.9 ± 0.9 (9.2-12.0)	34.1 ± 3.6 (27.4-40.3)	11.3 ± 1.3 (10.2-14)	39.2 ± 2.4 (10.2-14.0)	6.2 ± 0.3 (5.6-6.6)	32 ± 4.2 (25.3-38.1)
c’	4.2 ± 0.2 (3.9-4.6)	1.0 ± 0.1 (0.9-1.2)	4.7 ± 0.3 (4.3-5.2)	1.0 ± 0.1 (0.9-1.2)	7.3 ± 0.6 (6.8-8.9)	1.1 ± 0.1 (0.9-1.4)
Esophagus length	130 ± 9.3 (104.7-137.7)	137.5 ± 8.2 (122.3-146.7)	142 ± 7.4 (132-156.5)	142 ± 8.0 (137-163)	129.5 ± 3.3 (122-134)	107.3 ± 6.6 (94.5-115.7)
Head to nerve ring distance (NR)	98.2 ± 5.7 (84.6-105.8)	87.4 ± 6.2 (75.0-97.8)	99.4 ± 6.7 (86.4-106)	78.8 ± 12.4 (63.5-106)	93.0 ± 2.7 (89-97)	53.0 ± 4.0 (48.9-63.6)
Excretory pore position (EP)	66.5 ± 7.2 (60.0-84.3)	84.2 ± 9.1 (78.2-109.2)	63.5 ± 3.5 (58.7-71.0)	96.4 ± 9.1 (73.4-102.7)	115.5 ± 7.3 (101-126)	87.4 ± 10.3 (70.1-104.3)
Max body width	29.4 ± 1.7 (27.8-33.0)	61.8 ± 5.1 (53.8-68.5)	30.2 ± 1.9 (27.7-34.2)	114.5 ± 8.5 (101-126)	24.5 ± 1.1 (23.0-26.0)	47.4 ± 2.0 (44.0-50.5)
Anal body diam ABD	19.5 ± 2.0 (17.7-24.0)	33.3 ± 0.9 (32.6-35.0)	17.5 ± 1.4 (14.7-19.0)	38.3 ± 2.6 (36.0-42.4)	14.0 ± 1.0 (11.5-15.0)	24.7 ± 2.0 (22.0-27.7)
Tail length	82.7 ± 9.2 (72.4-98.0)	34.1 ± 4.0 (29.3-42.4)	81.7 ± 8.5 (63.5-93.0)	37.8 ± 3.9 (32.5-44.0)	102.5 ± 3.1 (99.5-109)	28.3 ± 3.6 (22.8-32.6)
Testis length	-	730 ± 75.7 (617-910)	-	1174 ± 89.5 (1062-1368)	-	565.0 ± 82.6 (352-637)
Spicules length	-	58.2 ± 6.1 (45.5-66.8)	-	70.7 ± 4.4 (63.5-78.0)	-	43.5 ± 3.3 (39.1-48.9)
Gubernaculum length	-	45.6 ± 3.3 (40.8-52.2)	-	55.6 ± 4.9 (45.6-62.0)	-	22.8 ± 3.7 (16.3-29.3)
T %	-	63.4 ± 5.6 (58.6-78.3)	-	76.9 ± 5.0 (73.6-89.6)	-	63.4 ± 9.0 (40.5-71.8)
D % (EP/Esophagus length)	51.5 ± 7.7 (45.6-66.3)	61.5 ± 8.6 (53.3-81.7)	45.0 ± 4.1 (37.5-53.7)	67.9 ± 7.1 (51.1-73.3)	89.2 ± 4.2 (78.9-94.0)	81.5 ± 8.3 (62.0-91.4)
E % (EP/Tail length)	80.7 ± 6.0 (69.3-90.7)	250 ± 39.5 (185-319)	75.7 ± 8.2 (69.6-94.9)	254 ± 23.6 (222-286)	112.7 ± 8.4 (99.0-126.0)	311.0 ± 35.1 (251.0-353.0)
SL/ABW	-	1.8 ± 0.2 (1.4-2.0)	-	1.9 ± 0.2 (1.6-2.1)	-	1.8 ± 0.2 (1.5-2.1)
GS % (GL/SL)	-	78.9 ± 7.2 (70.3-96.4)	-	78.7 ± 6.3 (65.1-85.7)	-	52.3 ± 6.8 (38.5-60.0)

Table 3 – Characteristics of soil samples relative to EPN and vegetation type.

Species name	Name of EPN sample	Texture	pH	Organic Matter (%)	Relative Humidity (%)
Heterorhabditis indica	AYAB6	Loamy sand	7.87	2.08	13.78003
Heterorhabditis indica	BRA20	Loamy sand	8.58	1.1	10.23936
Steinernema feltiae	ANFA5	Sandy loam	8.4	1.22	6.404782
Steinernema feltiae	EDA1	Sandy loam	7.93	3.72	5.29136
Oscheius sp.	BRA6	Sandy loam	7.6	6.11	9.803922

corresponding sequences of *S. feltiae* present in the database ranging from 0 to 4% (28-33 bp different). The sequencing of the 18S rRNA gene produced a sequence of 1435 bp. The partial 18S sequence was 99 % identical to *S. feltiae* (FJ040419), 1402/1404 identities and 0 gap, and 99% similar to *S. kraussei*, 1399/1406 identities and 2 gaps, that also belongs to the '*feltiae-kraussei-oregonense*' group. The amplification of the *mt*DNA COI from both isolates of *S. feltiae* produced a fragment of about 700 bp in length. The nucleotide sequences of *mt*DNA COI gene determined for both Lebanese *S. feltiae* isolates showed a 5% dissimilarity (607/639 identities) with each other, whereas at amino acid level they showed a 98% similarity (210/214 identities; 212/214 positives).

The sequences of the entire ITS of *H. indica* were determined for 2 specimens for each population (AYAB6 and BRA20) and it was 818 bp in length. A low intraspecific variability was observed among different clones of the same isolate and between isolates (4-6 bp different) that was identical to the corresponding sequences of *H. indica* present in the database. The sequences of the 18S rRNA gene produced a fragment of 1595 bp. No intraspecific variability was observed among specimens belonging to Lebanese isolates. The partial 18S sequence showed 97-98% similarity (27-44 bp different) with the corresponding sequences of *Heterorhabditis* species present in the database. The amplification of the *mt*DNA COI from two isolates of *H. indica* produced a fragment of 669 bp in length. No corresponding COI sequences of *H. indica* are present in the database.

The *Oscheius* strain (BRA 6) was isolated by using the *Galleria* trap method from a soil sample collected from agricultural habitat along the Lebanese coast. The death of *Galleria* larvae provides evidence for its entomopathogenic potential that must be further investigated.

The amplification of the ITS containing region of a hypothetical entomopathogenic nematode (BRA6 isolate) produced a fragment of 818 bp. Blast search at NCBI revealed that the ITS sequences matched well with few closely related *Oscheius* sequences, in particular with the corresponding region of *Oscheius* sp. MCB isolate (KF684370) showing a 99% similarity (798/810 identities, 1 gap) and with *Oscheius* sp. TEL-2014 isolated from *G. mellonella* larvae (KM492926) showing a similarity of 84% (665/788 identities and 33 gaps). The amplification of the *mt*DNA COI of *Oscheius* sp. from Lebanon produced a fragment of 666 bp. No corresponding COI sequences of *Oscheius* sp. are present in the database.

Phylogenetic relationships

Thirty-seven ITS sequences of *feltiae-kraussei-oregonense* isolates from all over the world including those from Lebanon, 9 of *Oscheius* spp. and eighteen of *Heterorhabditis* spp. were aligned. ML analysis generated a phylogenetic tree (Fig. I) containing five main groups: group I: *feltiae-kraussei-oregonense-ichnusae* sequences; group II: all *Heterorhabditis* species; group III: *Oscheius* sp. sequences including that from Lebanon; and group IV: *Oscheius tipulae*; group V: *O. carolinensis*, other *Oscheius* sequences that matched well (84% similarity) with the Lebanese one and *C. elegans* sequence as outgroup, at basal position of the tree. The monophyly of the group I and II was clearly supported, while *Oscheius* sequences clustered in different groups. In the group I was well supported the subdivision into three subgroups: 1) *S. feltiae-ichnusae*; 2) *S. kraussei*, 3) *S. oregonense*. In the second group, two subgroups are evident: the *indica*-subgroup (Nguyen *et al*., 2004; Andalò *et al*., 2006; Maneesakorn *et al*., 2011) and the *megidis*-subgroup (Phan *et al*., 2003; Maneesakorn *et al*., 2011). The *indica*-subgroup contained all sequences of *H. indica* including those from Lebanon and *H. sonorensis*, *H. taysearae*, *H. mexicana*, *H. floridensis* and *H. amazonensis*. The *megidis*-subgroup contained *H. downesi*, *H. megidis*, *H. marelatus* and *H. zealandica*. *Heterorhabditis bacteriophora* resulted closely related to *indica*-subgroup. The group III contained *Oscheius* sp. from Lebanon and sequences of *Oscheius* spp. and *O. onirici*. Group IV contained *O. tipulae*. In the group V clustered other *Oscheius* spp. together with *C. elegans*.

For phylogenetic analysis of the 18S rRNA gene, the corresponding sequences of 11 *Steinernema* spp. and 11 *Heterorhabditis* spp. were aligned in this study. In the ML tree (Fig. II) two monophyletic groups were supported. Group I clustered all sequences of *Steinernema* species including *S. feltiae* from Lebanon. Group II contained all sequences from *Heterorhabditis* species including *H. indica* sequences from Lebanon and the sequence of *Panagrellus redivivus* as outgroup.

For phylogenetic analysis of the mitochondrial COI gene, the aminoacid sequences of 4 *S. feltiae*, 2 of *S. carpocapsae* from Italy, 2 of *H. indica* from Lebanon, 2 of *H. bacteriophora*, 1 of *Oscheius* sp. from Lebanon, 1 of *O. onirici* and 1 of *C. elegans* were aligned. In the ML tree (Fig. III) *S. feltiae* isolates from Lebanon clustered together with the reference sequence of *S. feltiae* showing some diversity among isolates supported by bootstrap values that vary between 68 and 92%. The *S. feltiae* group resulted well separated from *Heterorhabditis* group confirming the different origin. *Oscheius* sp. from Lebanon clustered at the basal position in the tree with *O. onirici*, while *C. elegans* resulted no a good outgroup for COI phylogeny.

The phylogenetic tree reported in Fig. IV is based on the concatenation of partial sequences of five gene fragments *recA, dnaN, gltX, gyrB*and *infB*, comparing sequences of bacterial strains LB05, LB10 and LB06 with sequences of representative strains of the different species with the genus *Xenorhabdus* and *Photorhabdus*. All sequences showed a high percentage of homology (98-100 %) to the sequences derived from *X. bovienii* or from *P. luminescens subsp. akhurstii* suggesting that they belong to the same phylogenetic group, respectively.

DISCUSSION

The objective of this study was to isolate and characterize EPNs along the Lebanese coasts that have been so far under-explored, and also to describe their symbiotic bacteria and to determine species habitat preferences. A polyphasic approach, combining morphological and molecular datasets, has been proved to be more reliable for a robust identification of EPNs. Sequence analyses of the ITS, the partial 18S rRNA gene and the mitochondrial COI gene well supported the identification of *S. feltiae* and *H. indica* reported for the first time in Lebanon, and *Oscheius* sp. along Lebanese coasts. In a previous survey of EPNs in Lebanon, *S. feltiae* and *H. bacteriophora* were already found (Noujeim *et al*., 2011) and both species were also reported in other countries of the Mediterranean region (Hazir *et al.,* 2003; Valadas *et al*., 2013; Tarasco *et al*., 2015). *Steinernema feltiae* is known as the most widespread EPN species occupying every habitat and continent. *Heterorhabditis indica* is known to be a species that can tolerate high temperatures and has been associated with

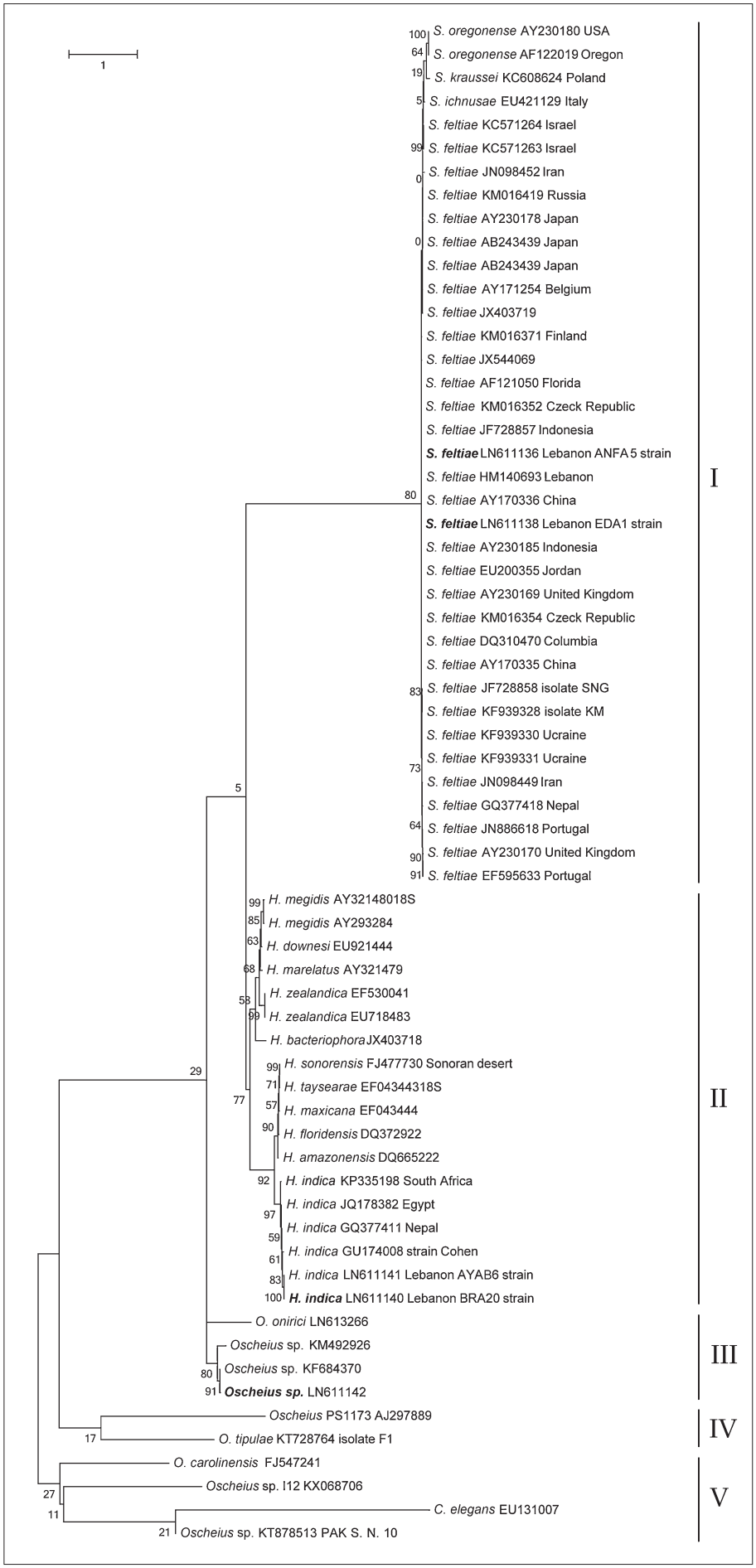

Fig. I – Phylogenetic tree of ITS containing region describing the evolutionary relationships among different species of *Heterorhabditis*, *Steinernema* and *Oscheius* sp. using Maximum Likely (ML) method. Branch lengths are proportional to the distances as derived from the distance matrix obtained using the GTR method with the invariant site plus gamma options. Bayesian 50% majority rule consensus trees as inferred from ITS sequences. Numbers at nodes indicate bootstrap values.

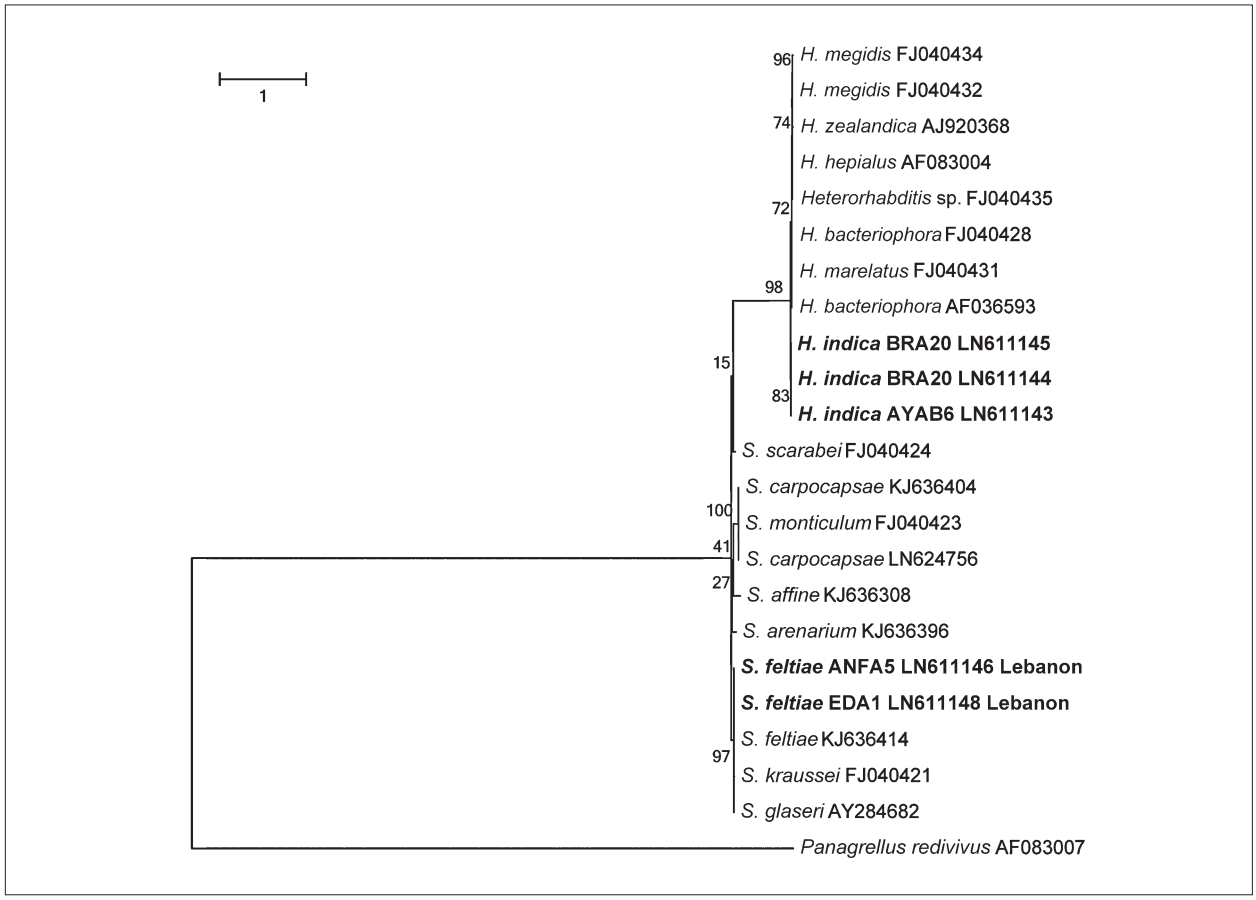

Fig. II – Phylogenetic tree of the 18 S rRNA gene sequences describing the evolutionary relationships among different species of *Heterorhabditis* and *Steinernema* using Maximum Likely (ML) method. Branch lengths are proportional to the distances as derived from the distance matrix obtained using the GTR method with the invariant site plus gamma options. Bayesian 50% majority rule consensus trees as inferred from 18S partial sequences. Numbers at nodes indicate bootstrap values.

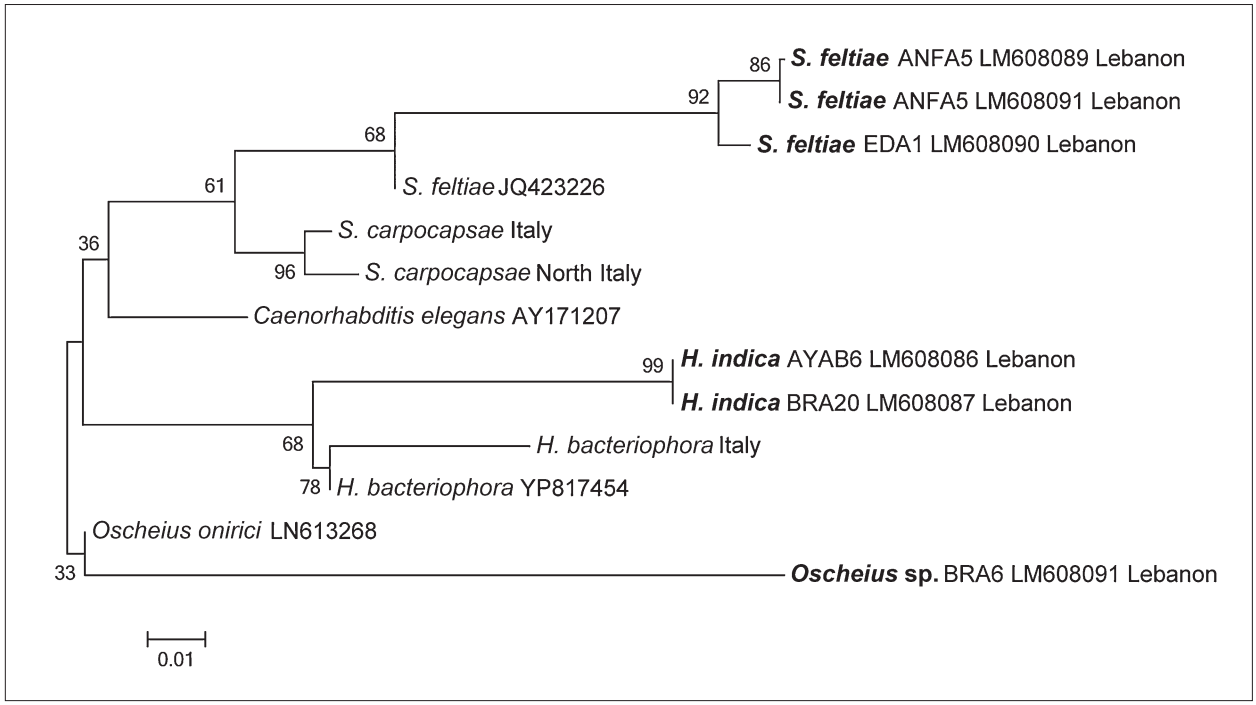

Fig. III – Phylogenetic tree of the mitochondrial COI aminoacid sequences describing the evolutionary relationships among different species of *Heterorhabditis*, *Steinernema* and *Oscheius* sp. using Maximum Likely (ML) method. Branch lengths are proportional to the distances as derived from the distance matrix obtained using the GTR method with the invariant site plus gamma options. Bayesian 50% majority rule consensus trees as inferred from mitochondrial COI sequences. Numbers at nodes indicate bootstrap values.

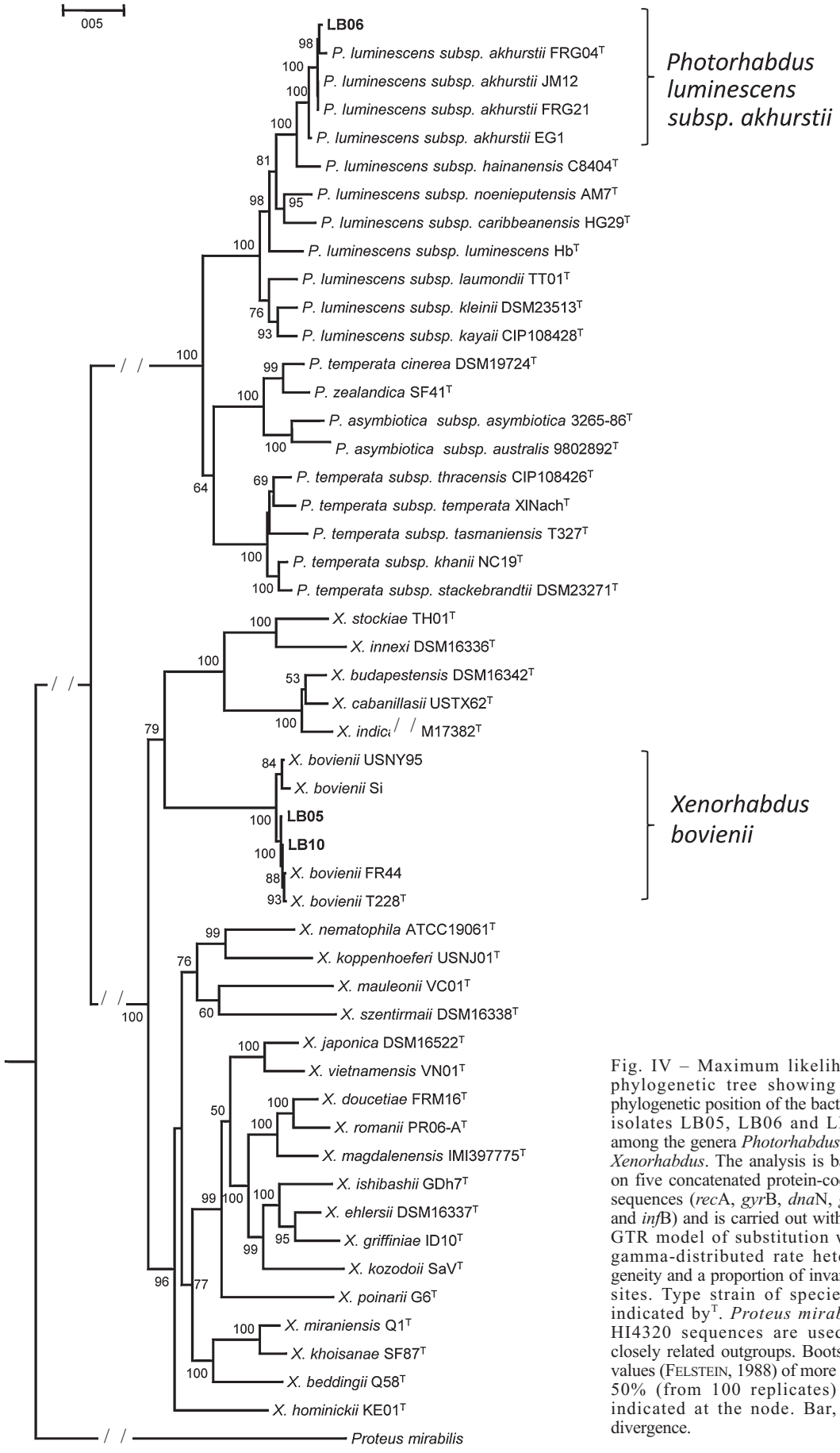

Fig. IV – Maximum likelihood phylogenetic tree showing the phylogenetic position of the bacterial isolates LB05, LB06 and LB10 among the genera *Photorhabdus* and *Xenorhabdus*. The analysis is based on five concatenated protein-coding sequences (*rec*A, *gyr*B, *dna*N, *glt*X and *inf*B) and is carried out with the GTR model of substitution with gamma-distributed rate heterogeneity and a proportion of invariant sites. Type strain of species is indicated by^T. *Proteus mirabilis* HI4320 sequences are used as closely related outgroups. Bootstrap values (FELSTEIN, 1988) of more than 50% (from 100 replicates) are indicated at the node. Bar, 5% divergence.

sandy coastal soils and away from coast lines (PHAN *et al*., 2003; DOLINSKI *et al*., 2008). It was first described from India and in the Southern side of Lebanon, *H. indica* was isolated in agricultural fields cultivated with banana and orange. This coastal area is characterized by hot and humid summers representing favorable conditions for this nematode. Despite being isolated in agricultural fields, *H. indica* is rarely used in biological control and it will be interested to try to valorize this specie to control pests in Lebanon. In the current survey in Lebanon, no EPNs were recovered from the beaches, as reported in literature (PHAN *et al*., 2001; MAULEON *et al*., 2006; TARASCO *et al*., 2008, 2015; EDGINGTON *et al*., 2010). In fact, the coastline in Lebanon undergoes a serious ecological pressure mainly due to the anarchic urbanization, destruction and permanent construction of buildings and resorts and sand extractions. In addition habitats of seabed are severely destructed by various pollutants which might affect negatively the presence of EPNs. Additional studies might be needed to evaluate the impact of different pollutant on the presence of EPNs.

Our phylogenetic analyses based on the ITS sequences, the 18S rRNA gene and *mt*COI firmly confirmed the different monophylies of *Heterorhabditis*, *Steinernema* and *Oscheius* species showing that they evolved from different ancestors and that similarities of the life cycles can be attributed to convergence. The high intra-specific sequence variability of the *S. feltiae* ITS can be explained as the result of different rate of ITS evolution in *S. feltiae* that is not due to geographical origin because isolates from the same region did not cluster together and may also derive from the diverse soil types and insect hosts. Thus, heterogeneity of ITS did not preclude species discrimination of *S. feltiae* and the subdivision in *feltiae-kraussei-oregonense* subgroup.

Heterorhabditis indica isolates showed very low intra-specific variability of ITS sequences forming a well-supported cluster including those from Lebanon.

The phylogenetic relationships of *Oscheius* isolates (Fig. I) revealed that the ITS sequences of the *Oscheius* sp. BRA6 isolate from Lebanon clustered with ITS sequences of *Oscheius* species (GenBank accession number KF684370 and KM492926). One of them, *Oscheius* sp.TEL-2014 isolate was also recovered from *Galleria* larvae and recently confirmed as entomopathogenic nematode (LEPHOTO *et al*., 2016). So far only three species belonging to *Oscheius* genus, *O. carolinensis*, *O. chongmingensis* and *O. onirici* were demonstrated to be entomopathogenic nematodes (YE *et al*., 2010; TORRES-BARRAGAN *et al*., 2011; LIU *et al*., 2012; TORRINI *et al*., 2015).

Figure II shows a phylogenetic tree based on the nearly full-length 18S rDNA in which the different monophyly of *Heterorhabditis* and *Steinernema*, including those from Lebanon is confirmed. Some species belonging to both genera are not well resolved suggesting that they may have diverged from each other relatively recently. In our phylogenetic analysis *S. monticolum* resulted closely related to *S. carpocapsae* (NADLER *et al*., 2006) than to *S. feltiae* as recently reported by DILLMAN *et al*., 2015. The position of *S. glaseri* is not well resolved resulting closely related to *S. feltiae*. These results clearly suggest that the 18S fragment used in this study is very useful to resolve the phylogenetic relationships among *Steinernema* and *Heterorhabditis* species.

The phylogenetic relationships by using the COI sequences confirmed the different monophyly of *Heterorhabditis*, *Steinernema* and *Oscheius* sp. Nevertheless, even COI accumulates substitutions more quickly than ITS, it results a useful marker for differentiating geographical isolates of EPN. The variation of the aminoacid sequences of *mt*COI between individuals of the same species reaches up to 2%, as found for other species of nematodes (BLOUIN *et al*., 1998).

Our characterization study of the bacterial symbiont datasets have demonstrated the phylogenetic congruence between *H. indica* and the specific symbiotic bacteria *P. luminescens* subs. *akhurstii* and between *S. feltiae* and the specific symbiotic bacteria *X. bovienii*, as reported in literature (MANEESAKORN *et al*., 2011; THANWISAI *et al*., 2012).

In conclusion this survey of soils along to Lebanese coasts shows that EPNs are not present in soil beaches but they are present in different agricultural habitats close to the beaches as a result of high anthropogenic activities and pollution. Furthermore, the global distribution throughout the world and the high ITS sequence variability of *S. feltiae* among all populations included in this study suggest a range of phenotypic characters that give it an advantage to adapt in different habitats. In addition *H. indica*, recorded for the first time in Lebanon, shows very little diversity among the populations studied and an affinity to sandy soils suggesting limited tolerance to prolonged cool conditions. The isolation of a potential EPN belonging to the genus *Oscheius* needs further investigations in order to reveal the mechanism of pathogenicity in this nematode and to assess potential applications for pest management.

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REFERENCES

ALTSCHUL S.F., MADDEN T.L., SCHÄFFER A.A., ZHANG J., ZHANG Z., MILLER W., LIPMAN D.J., 1997 – *Gapped BLAST and PSI-BLAST: a new generation of protein database search programs*. - Nucleic Acids Research, 25: 3389-3402.

ANDALÓ V., NGUYEN K.B., MOINO A., 2006 – Heterorhabditis amazonensis *n.sp. (Rhabditida: Heterorhabditidae) from Amazonas, Brazil*. - Nematology, 8: 853-867.

BEDDING R.A., AKHURST R.J., 1975 – *A simple technique for the detection of insect parasitic rhabditid nematodes in soil*. - Nematologica, 21: 109-110.

BOEMARE N., AKHURST R.J., MOURANT R.J., 1993 – DNA *relatedness between* Xenorhabdus *spp. (Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes and a proposal to transfer* Xenorhabdus luminescens *to a new genus,* Photorhabdus *gen. nov*. - International Journal of Systematic Bacteriology, 43: 249-255.

BOEMARE N., THALER J.O., LANOIS A., 1997 – *Simple bacteriological tests for phenotypic characterization of* Xenorhabdus *and* Photorhabdus *phase variants*. - Symbiosis, 22: 167-175.

CASTRESANA J., 2000 – *Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis*. - Molecular Biology and Evolution, 17: 540-552.

DE LUCA F., FANELLI E., DI VITO M., REYES A., DE GIORGI C., 2004 – *Comparison of the sequences of the D3*

expansion of the 26S ribosomal genes reveals different degrees of heterogeneity in different populations and species of Pratylenchus *from the Mediterranean region*. - European Journal of Plant Pathology, 111: 949-957.

Dereeper A., Guignon V., Blanc G., Audic S., Buffet S., Chevenet F., Dufayard J.F., Guindon S., Lefort V., Lescot M., Claverie J.M., Gascuel O., 2008 – *Phylogeny for: robust phylogenetic analysis for the non-specialist*. - Nucleic Acids Research*.*, 2008 1, 36 (*Web Server issue*), W 465-9.

Dillman A.R., Macchietto M., Porter C.F., Rogers A., Williams B., Antoshechkin I., Lee M.M., Goodwin Z., Lu X., Lewis E.E., Goodrich-Blair H., Stock S.P., Adams B.J., Sternberg P.W., Mortazavi A., 2015 – *Comparative genomics of* Steinernema *reveals deeply conserved gene regulatory networks.* - Genome Biology, 16: 200-221.

Dolinski C., Kamitani F.L., Machado I.R., Winter C.E., 2008 – *Molecular and morphological characterization of heterorhabditid entomopathogenic nematodes from the tropical rainforest in Brazil*. - Memórias do Instituto Oswaldo Cruz, Rio de Janeiro, 103: 150-159.

Edgar R.C., 2004 – MUSCLE: *multiple sequence alignment with high accuracy and high throughput*.- Nucleic Acids Research, 32: 1792-1797.

Edgington S., Buddie A.G., Moore D., France A., Merino L., Tymo L.M., Hunt D.J., 2010 - *Diversity and distribution of entomopathogenic nematodes in Chile*. - Nematology, 12: 915-928.

Felsenstein J., 1988 – *Phylogenies from molecular sequences: inference and reliability*.- Annual Review of Genetics, 22: 521-565.

Forst S., Clarke D., 2002 – *Bacteria-nematode symbiosis*. In: Entomopathogenic Nematology. Gaugler R. Ed., - CABI, London, pp. 57-77.

Grant J.A., Villani M.G., 2003a – *Soil moisture effects on entomopathogenic nematodes*. - Environmental Entomology, 32: 80-87.

Grant J.A., Villani M.G., 2003b – *Effects of soil rehydration on the virulence of entomopathogenic nematodes*. - Environmental Entomology, 32: 983-991.

Hazir S., Keskin N., Stock P.S., Kaya H.K., Özcan S., 2003 – *Diversity and distribution of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Turkey*. - Biodiversity and Conservation, 12: 375-386.

Hominick W.M., 2002 – *Biogeography*. In: Entomopathogenic Nematology. Gaugler R. Ed., CABI, Wallingford, Oxon, UK, pp. 115-143.

Hooper D.J., 1970 – *Handling, fixing, staining and mounting nematodes*. In: Laboratory Methods for Work with Plant and Soil Nematodes. Southey J. Ed., Ministry of Agriculture*,* Fisheries and Food. - Technical Bulletin 2, London, UK*,* pp. 39-54.

Kanzaki N., Futai K., 2002 – *A PCR primer set for determination of phylogenetic relationships of* Bursaphelenchus *species within* xylophilus *group*. - Nematology, 4: 35-41.

Kaya H.K,. Stock S.P., 1997 – *Techniques in insect nematology*. In: Manual of Techniques in Insect Pathology. Lacey L.A. Ed. - Academic Pr*ess,* London*,* pp. 281-324.

Koppenhofer A.M., Kaya H.K., Taormino S.P., 1995 – *Infectivity of entomopathogenic nematodes (Rhabditida: Steinernematidae) at different soil depths and moistures*. - Journal of Invertebrate Pathology, 65: 193-199.

Kung S., Gaugler R,. Kaya H.K., 1991 – *Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence*. - Journal of Invertebrate Pathology, 57: 242 -249.

Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M. Wilm A., Lopez R., Thompson J.D., Gibson T.J., Higgins D.G., 2007 – *ClustalW and ClustalX version 2*. - Bioinformatics, 23 (21): 2947-2948.

Lephoto T.E., Mpangase P.T., Aron S., Gray V.M., 2016 – *Whole genome sequence of* Oscheius *sp. TEL-2014 entomopathogenic nematodes isolated from South Africa*. - Genomics Data, 7: 259-261.

Liu Q., Mracek Z., Zhang L., Puza V., Dong L., 2012 – *Re-description of* Oscheius chongmingensis *(Zhang et al., 2008) (Nematoda: Rhabditidae) and its entomopathogenicity*. - Nematology, 14: 139-149.

Maneesakorn P., An R., Daneshvar H., Taylor K., Bai X., Adams B.J., Grewal P. S., Chandrapatya A., 2011 – P*hylogenetic and cophylogenetic relationships of entomopathogenic nematodes (Heterorhabditis: Rhabditida) and their symbiotic bacteria* (*Photorhabdus*: *Enterobacteriaceae*). - Molecular Phylogenetics and Evolution, 59: 271-280.

Mauléon H., Denon D., Briand S., 2006 – *Spatial and temporal distribution of* Heterorhabditis indica *in their natural habitats in Guadalupe*. - Nematology, 8: 603-617.

Molyneux A.S., Bedding R.A., 1984 – *Influence of soil texture and moisture on the infectivity of* Heterorhabditis *sp. D1 and* Steinernema glaseri *for larvae of the sheep blowfly* Lucilia cuprina. - Nematologica, 30: 358-365.

Nadler S.A., Bolotin E., Stock S.P., 2006 – *Phylogenetic relationships of* Steinernema *Travassos, (Nematoda: Cephalobina: Steinernematidae) based on nuclear, mitochondrial and morphological data*. - Systematic Parasitology, 63: 161-181.

Noujeim E., Khater C., Pages S., Ogier J.C., Tailliez P., Hamze M., Thaler O., 2011 – *The first record of entomopathogenic nematodes (Rhabditidae: Steinernematidae and Heterorhabditidae) in natural ecosystems in Lebanon: A biogeographic approach in the Mediterranean region*. - J. Invertebrate Pathology, 107: 82-85.

Nguyen K.B., Shapiro-Ilan D.J., Stuart R.J., Jame R.R., Adams B.J., 2004 – Heterorhabditis mexicana *n. sp. (Rhabditida: Heterorhabditidae) from Tamaulipas, Mexico, and morphological studies of the bursa of* Heterorhabditis *spp*. - Nematology, 6: 231-244.

Phan L.K., Subbotin S.A., Nguyen C.N., Moens M., 2003 – Heterorhabditis baujardi *sp. n. (Rhabditida: Heterorhabditidae) from Vietnam with morphometric data for* H. indica *populations*. - Nematology, 5: 367-382.

Phan L.K., Nguyen C.N., Moens M., 2001 – Steinernema loci *sp. n. and* Steinernema thanhi *sp. n. (Rhabditida: Steinernematidae) from Vietnam*. - Nematology, 3: 503-514.

Posada D., Crandall K.E., 1998 – *Modeltest: testing the model of DNA substitution*. - Bioinformatics, 14: 817-818.

Stock S.P., Campbell J.F., Nadler S.A., 2001 – *Phylogeny of* Steinernema *Travassos, 1927 (Cephalobina: Steinernematidae) inferred from ribosomal DNA sequences and morphological characters*.- Journal of Parasitology, 87: 877-889.

Subbotin S.A., Vierstraete A., De Ley P., Rowe J., Waeyenberge L., Moens M., Vanfleteren J.R., 2001 – *Phylogenetic relationships within the cyst-forming nematodes (Nematoda, Heteroderidae) based on analysis*

of sequences from the ITS regions of ribosomal DNA. - Molecular Phylogenetics and Evolution., 21: 1-16.

Tailliez P., Laroui C., Ginibre N., Paule A., Pagès S., Boemare N., 2010 – *Phylogeny of* Photorhabdus *and* Xenorhabdus *based on universally conserved protein-coding sequences and implications for the taxonomy of these two genera. Proposal of new taxa*: X. vietnamensis *sp. nov*., P. luminescens *subsp*. caribbeanensis *subsp. nov*., P. luminescens *subsp*. hainanensis *subsp. nov*., P. temperata *subsp.* khanii *subsp. nov*., P. temperata *subsp*. tasmaniensis *subsp. nov*., *and the reclassification of* P. luminescens *subsp*. thracensis *as* P. temperata *subsp.* thracensis. - International Journal of Systematic and Evolutionary Microbiology, 60: 1921-1937.

Tailliez P., Pagès S., Edgington S., Lukasz M.T., Buddie A.G., 2012 – *Description of* Xenorhabdus magdalenensis *sp. nov., the symbiotic bacterium associated with* Steinernema australe. - International Journal of Systematic and Evolutionary Microbiology, 62: 1761-1765.

Tamura K., Dudley J., Nei M., Kumar S., 2007 – *MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0*. - Molecular Biology and Evolution, 24: 1596-1599.

Tarasco E, Mrăcek Z, Nguyen K., Triggiani O., 2008 – Steinernema ichnusae *sp. n. (Nematoda: Steinernematidae) a new entomopathogenic nematode from Sardinia Island (Italy)*. - Journal of Invertebrate Pathology, vol. 99, p. 173-185, ISSN: 0022-2011

Tarasco E., Clausi M., Rappazzo G., Panzavolta T., Curto G., Sorino R., Oreste M., Longo A., Leone D., Tiberi R., Vinciguerra M.T., Triggiani O., 2015 – *Biodiversity of entomopathogenic nematodes in Italy*. - Journal of Helminthology, 89: 359-366

Thanwisai A., Tandhavanant S., Saiprom N., Warefield N.R., Long P.K., Bode H.B., Peacock S.J., Chantratita N., 2012 – *Diversity of* Xenorhabdus *and* Photorhabdus *spp. and their symbiotic entomopathogenic nematodes from Thailand*.- Plos One, 7: e43835.

Thomas G.M., Poinar G.O. Jr., 1979 – Xenorhabdus *gen. nov., a genus of entomopathogenic, nematophilic bacteria of the family Enterobacteriaceae*. - International Journal of Systematic Bacteriology, 29: 352-360.

Thomas G.W., 1996 – *Soil pH and Soil Acidity*. In: Methods of Soil Analysis: Chemical Methods. Part 3. Sparks D.L. Ed., - Soil Science Society of America Journal, Madison WI.

Torres-Barragan A., Suazo A., Buhler W.G., Cardoza Y.J., 2011 – *Studies on the entomopathogenicity and bacterial associates of the nematode* Oscheius carolinensis. - Biological control, 59: 123-129.

Torrini G., Mazza G., Carletti B., Benvenuti C., Roversi P.F., Fanelli E., De Luca F., Troccoli A., Tarasco E., 2015 – Oscheius onirici *sp. n. (Nematoda: Rhabditidae): a new entomopathogenic nematode from an Italian cave*. - Zootaxa, 3937 (3): 533-48.

Valadas V., Laranjo M., Mota M., Oliveira S., 2013 – *A survey of entomopathogenic nematode species in continental Portugal*. - Journal of Helminthology. doi:10.1017/S0022149X13000217.

Wouts W.M., Mracek Z., Gerdin S., Bedding R.A., 1982 – Neoplectana *Steiner, 1929 a junior synonym of* Steinernema *Travassos, 1927 (Nematoda: Rhabditida)*. - Parasitology, 4: 147-154.

Ye W., Torres-Barragan A., Cardoza Y.J., 2010 – Oscheius carolinensis *n. sp. (Nematoda: Rhabditidae), a potential entomopathogenic nematode from vermicompost*.- Nematology, 12: 121-135.